

Figure 1. Hypersensitivity of Gyrase Mutant Alleles

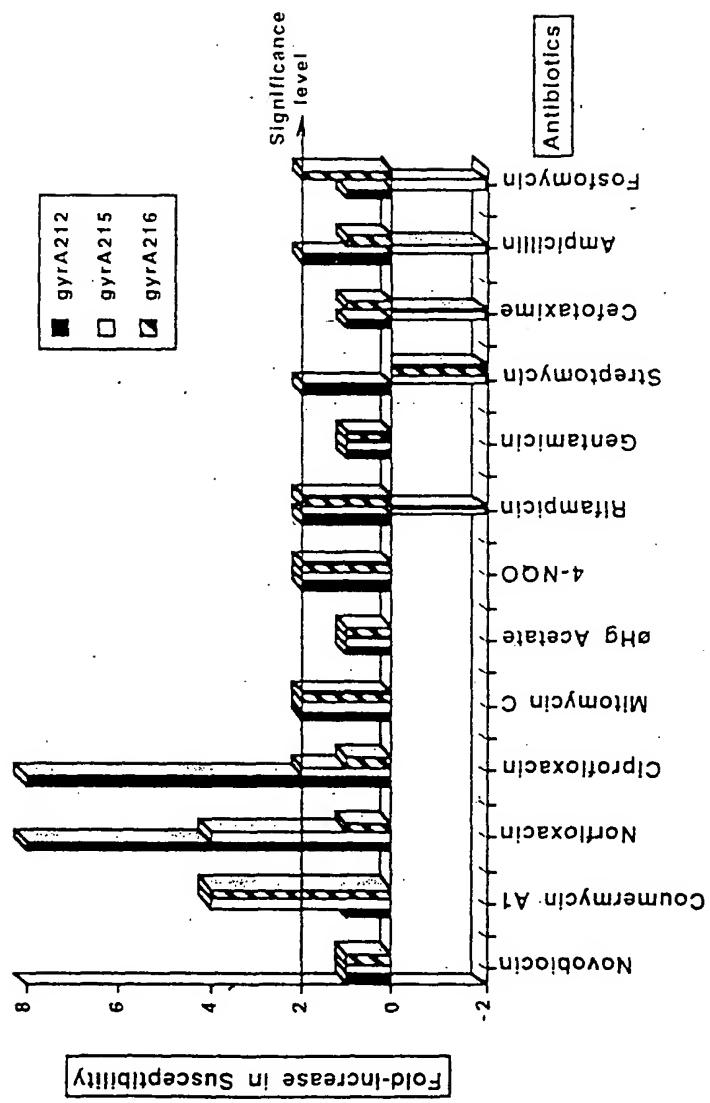


Figure 2. Hypersensitivity Profiles of *Salmonella* Ts Mutants

	Gyrase inhibitors		DNA/RNA metabolism				Protein metabolism			Cell wall inhibitors		
	Nov	Cou	Cipro	Nor	MitoC	NQO	Rif	Gen	Strep	Phen	Cefo	Amp
5155	dnaE	-	-	-	-	-	-	-	-	-	-	4
	gyrA216	-	-	-	-	-	-	-	-	-	-	-
	gyrA215	-	-	-	-	-	-	-	-	-	-	-
	gyrA212	-	-	-	-	-	-	-	-	-	-	-
7784	parC	-	-	-	-	-	-	-	-	-	-	-
	clm?	-	-	-	-	-	-	-	-	-	-	-
	parE	-	-	-	-	-	-	-	-	-	-	-
	parE	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	parF	-	-	-	-	-	-	-	-	-	-	-
	clm?	-	-	-	-	-	-	-	-	-	-	-
	murB	-	-	-	-	-	-	-	-	-	-	-
	murB	Round	-	-	-	-	-	-	-	-	-	-
7587	dapA	-	-	-	-	-	-	-	-	-	-	-
5118	murB	232	22645	22645	-	21645	22645	22645	22645	22645	22645	22645
5091	Thy Inc.	2842	325645	-	-	2842	325645	325645	325645	325645	325645	325645
7585	Odd	-	-	-	-	-	-	-	-	-	-	-
5208	fisH	-	-	-	-	-	-	-	-	-	-	-
7141	Filam	-	-	-	-	-	-	-	-	-	-	-
5052	Filam	-	-	-	-	-	-	-	-	-	-	-
5051	Filam	-	-	16	-	64	4	-	-	-	-	+4
5041	UV-	-	-	-	16	4	4	-	-	-	-	-
5086	UV-	-	4	-	+4	-	32	4	-	-	-	+32
	clm?	-	-	-	-	-	-	-	-	-	-	+8

* indicates that there was no significant difference with the wild type parent strain. *ND*: Not determined.

Abbreviations: Nov, novobiocin; Cou, coumermycin; Cip, ciprofloxacin; Nor, norfloxacin; MitC, mitomycin C; Cltg, phenylmercuric acetate; NQO, 4-nitroquinoline oxide; Rif, rifampicin; Gcn, gentamicin; Strep, streptomycin; Phen, phenol; Cef, ceftazidime; Amp, ampicillin; Fosf6, fosfomycin; clm?, unknown conditional lethal mutant; Round, round cell morphology; Thy inc., defective thymidine incorporation phenotype; Odd, odd cell shape morphology; Filam, filamentous cell morphology; UV-, UV sensitive. Known or closely related genotypes: *thrA*, DNA polymerase III α subunit; *gyrA*, gyrase subunit A; *parC*, Gyrb-like subunit of Topoisomerase IV; *parE*, GyrB-like subunit of Topoisomerase IV; *parF*, Acetyl transferase activity, associated with the topoisomerase IV gene; *murB*, UDP-N-acetylglucosaminyl-3-enolpyruvate reductase; *dapA*, Dihydrodipicolinate synthase; *nurCEFG*, near cluster of L-Ala, DAP, D-Ala-D-Ala and NAG ligases; *fisH*, maybe *fisH* by map location.

Figure 3. Potential Relationships Among Essential Genes

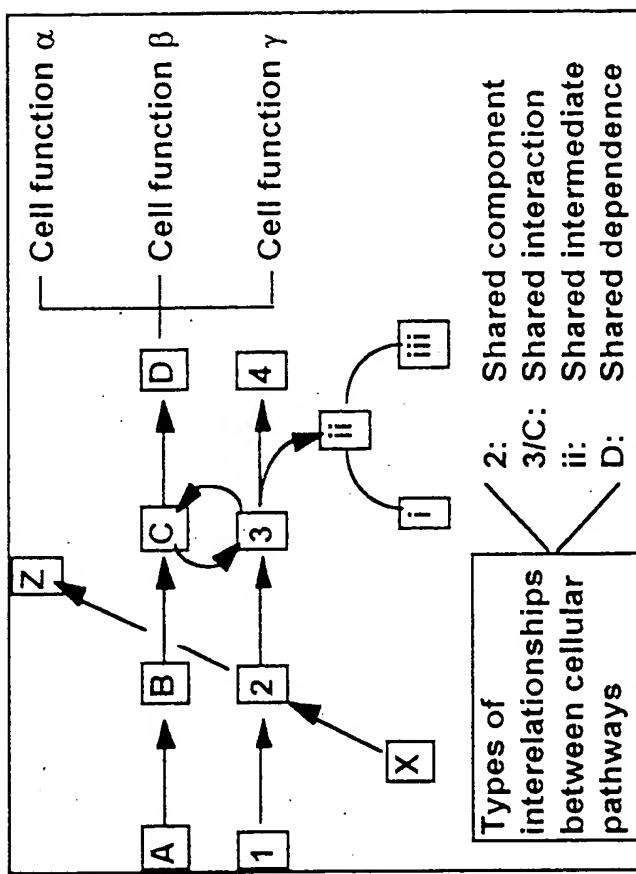


Figure 4. Single and Multi-Score Multichannel Screen

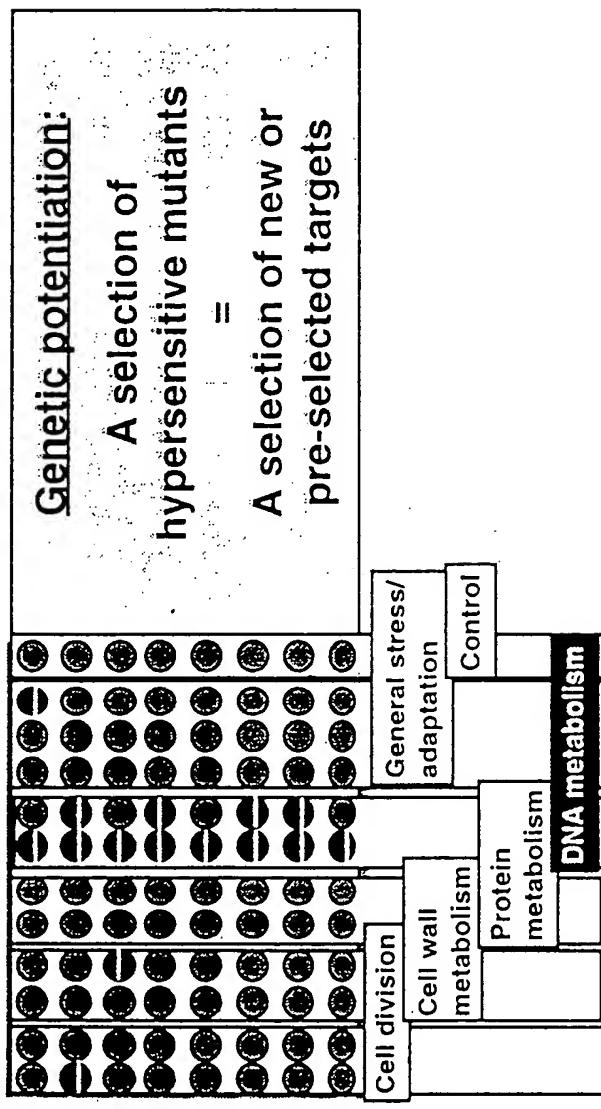


Figure 5. Operational Design of Multichannel Screen

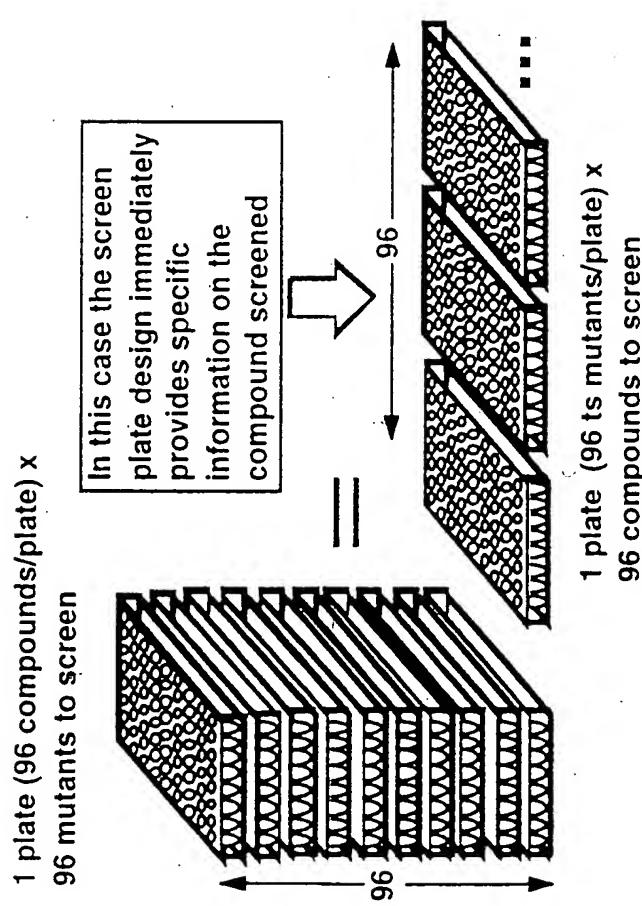


Fig 6

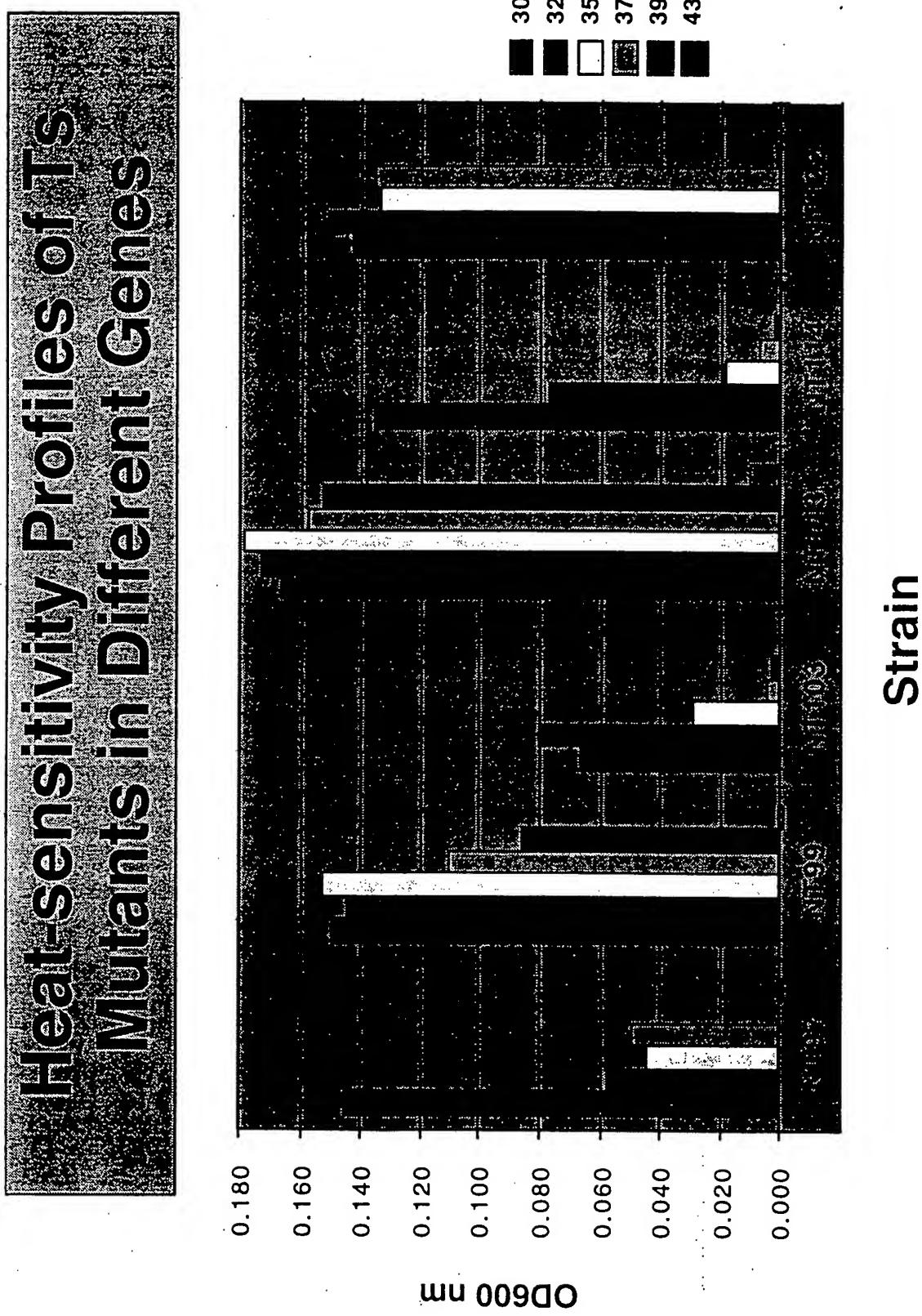
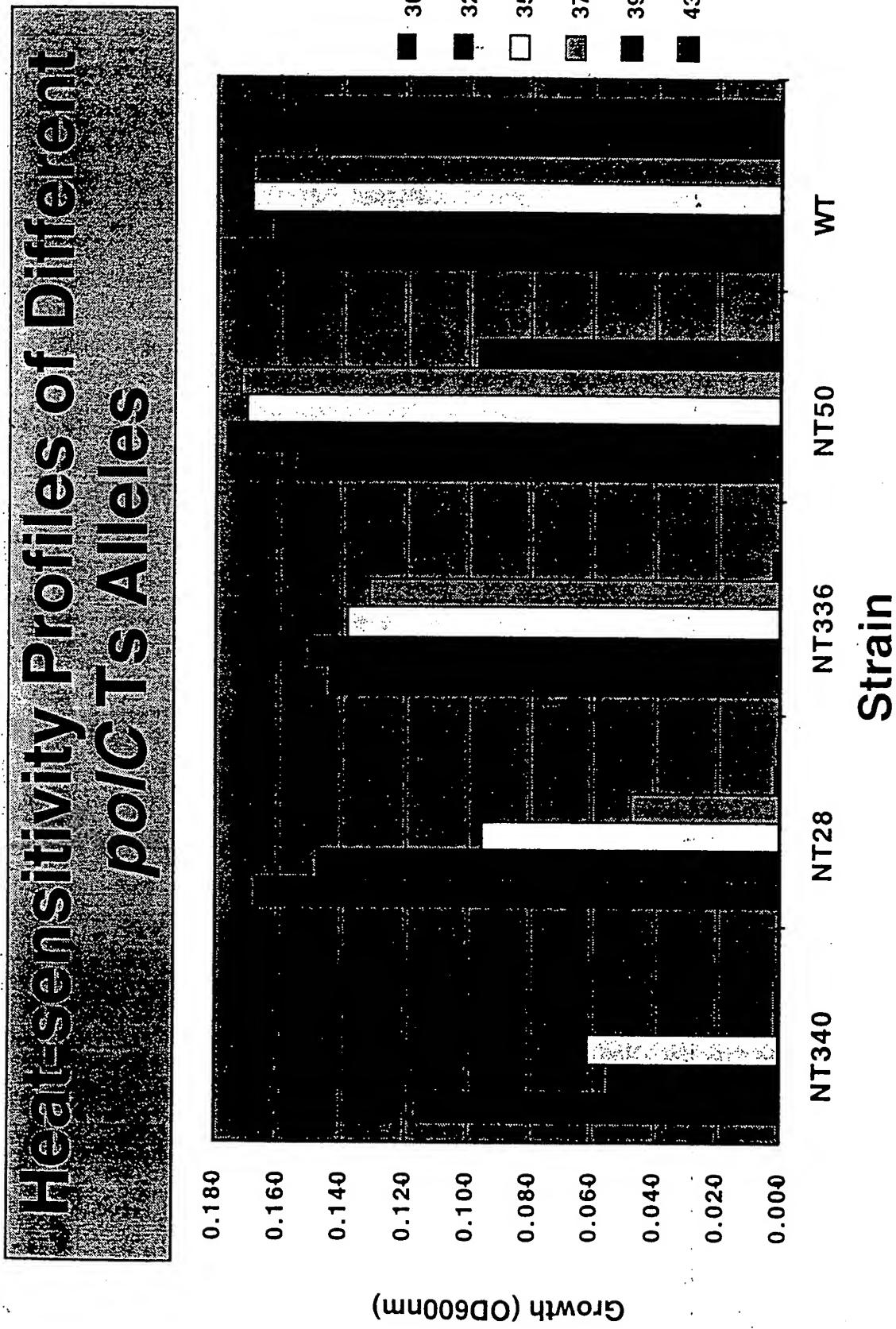


Fig 7

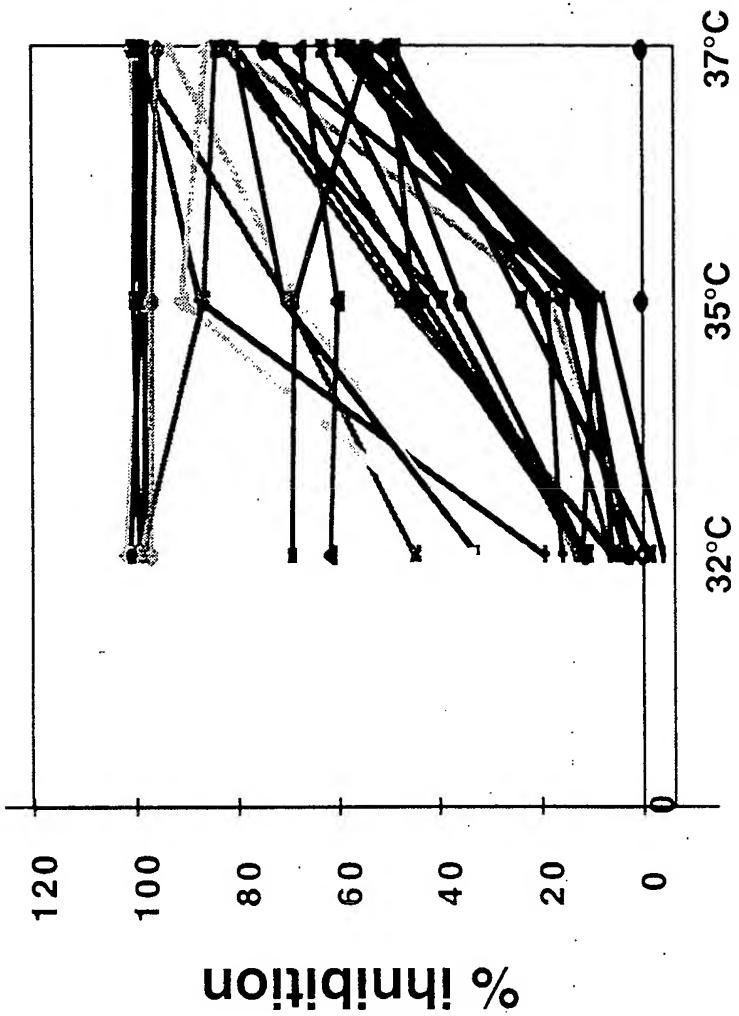


188

Temperature-dependent Hypersensitivity

Strain: NT 99

Inhibitory effect of
30 compounds at
 32°C , 35°C & 37°C

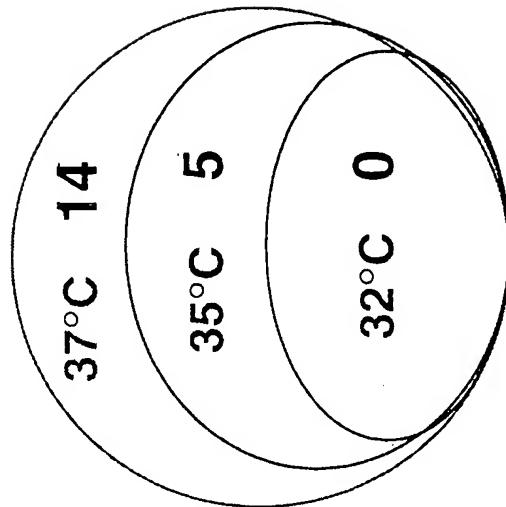


45°C

Fig 9

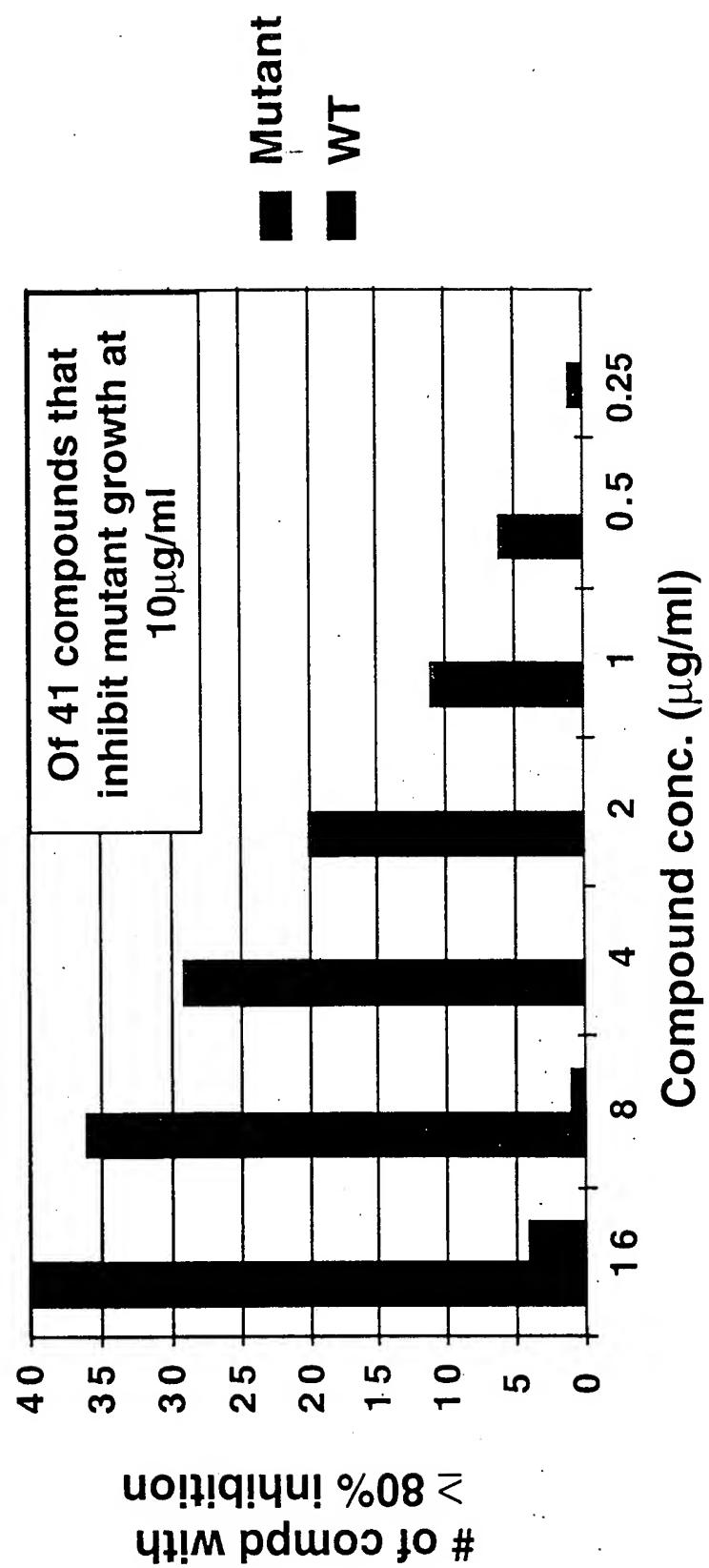


Hit criteria: compd that inhibits mutant by $\geq 50\%$, and % inh.
on mutant is higher than on WT by $\geq 30\%$
of compounds tested: 480 for NT99; 240 for NT340

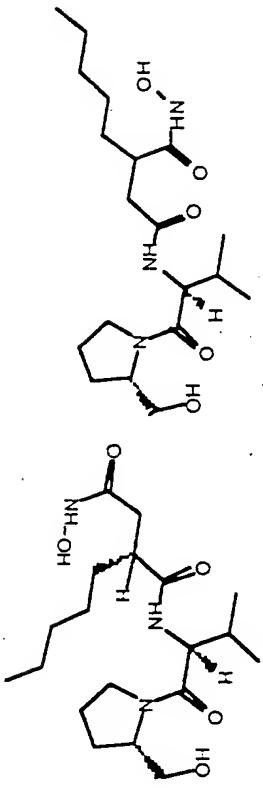


F18 10

Concentration Control of Hit Rate



T-18 // Structural Similarity of Compounds having
Identical Mutant Inhibition Profiles.



30-0014

20-0348

F18 12 Pilot Screen With Unknown Inhibitors Against *S. aureus**

	20. NT	10. 0157	50. 0617	20. 0116	20. 0204	20. 0860	20. 0123	10. 0287	10. 0045	10. 0373	10. 0752	10. 0197	10. 0374	10. 0797	10. 3775	10. 9370
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																
12																
13																
14																
15																
16																
17																
18																
19																
20																
21																
22																
23																
24																
25																
26																
27																
28																
29																
30																
31																
32																
33																
34																
35																
36																
37																
38																
39																
40																
41																
42																
43																
44																
45																
46																
47																
48																
49																
50																
51																
52																
53																
54																
55																
56																
57																
58																
59																
60																
61																
62																
63																
64																
65																
66																
67																
68																
69																
70																
71																
72																
73																
74																
75																
76																
77																
78																
79																
80																
81																
82																
83																
84																
85																
86																
87																
88																
89																
90																
91																
92																
93																
94																
95																
96																
97																
98																
99																
100																
101																
102																
103																
104																
105																
106																
107																
108																
109																
110																
111																
112																
113																
114																

*ND: No data available; blank boxes show no significant difference in MIC from the wild type strain (significance level > +/- 2-fold).

Figs 13

Anticipated Mutant Inhibition Profiles

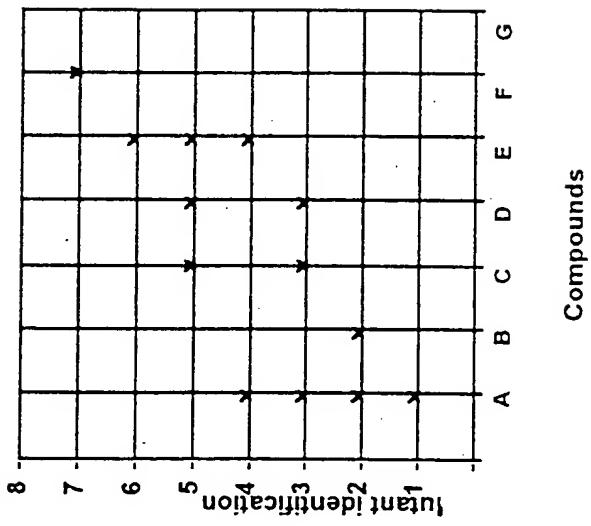
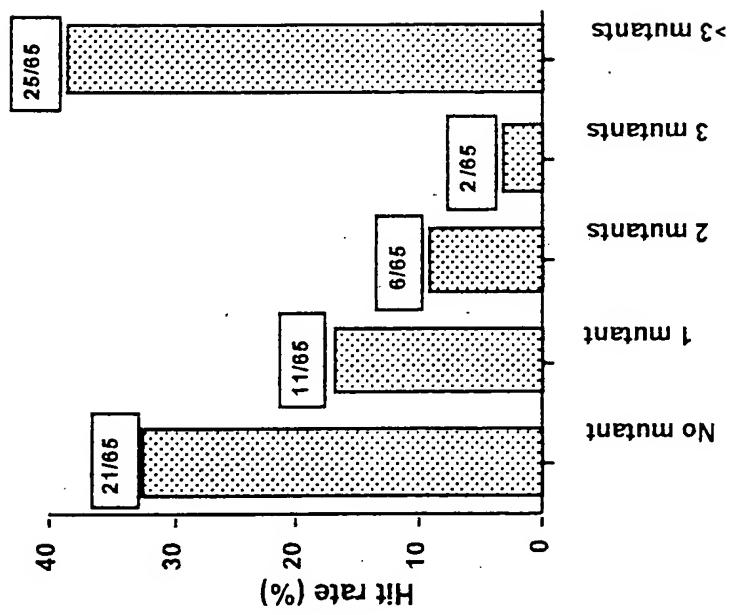


Fig 14 Hit Rate per Multiple of Mutants



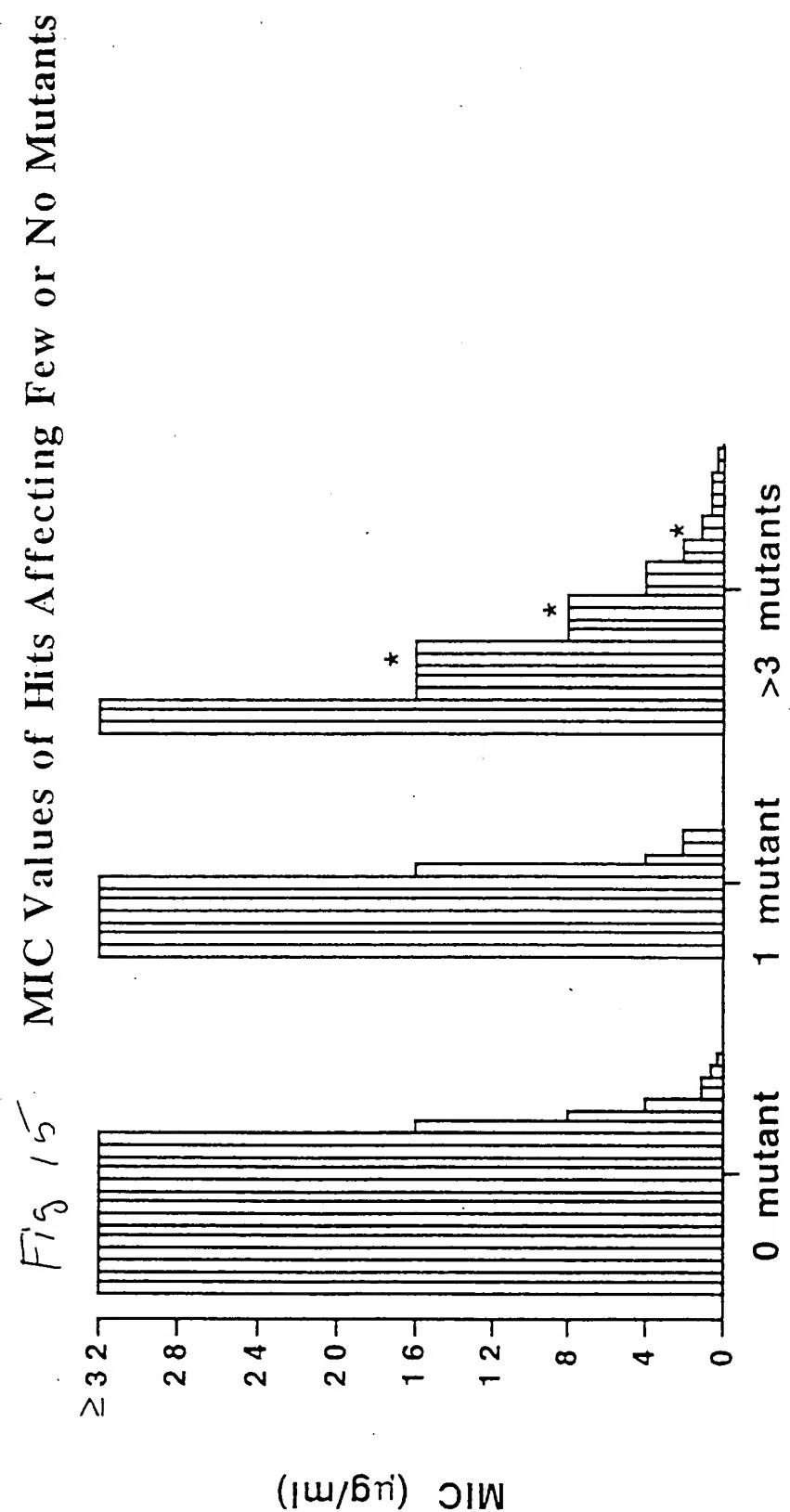
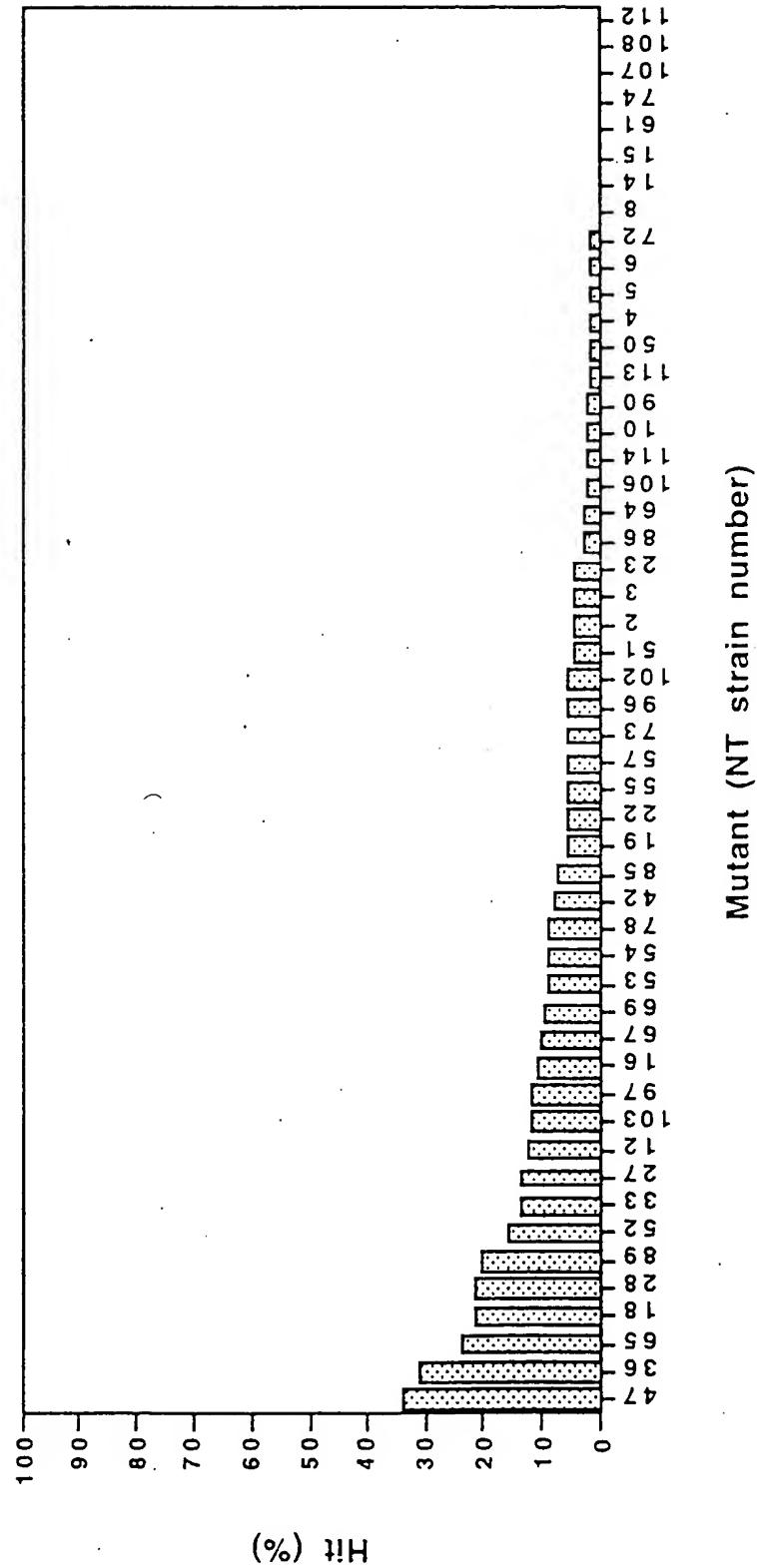


Fig. 16 Relative Number of Hits per Mutant



Fri /7 Multichannel Screen Advantage

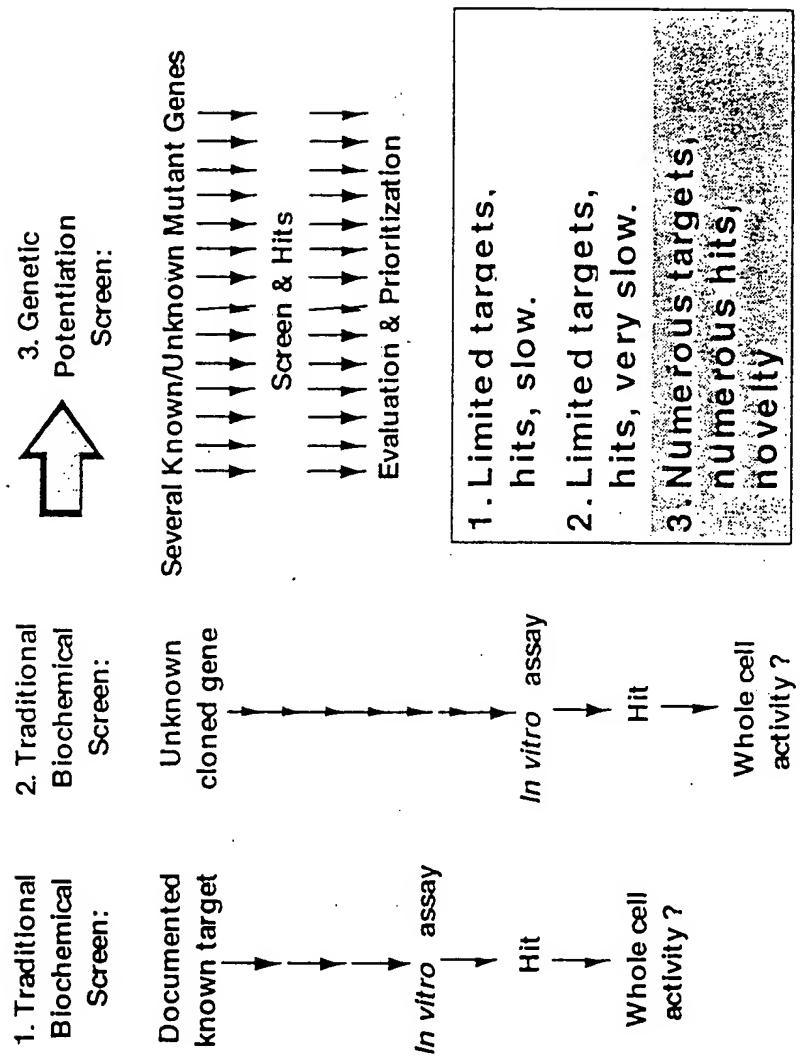


Fig 18 Strategy for Selection of Dominant Lethal Mutants

Relevant Genotype	Phenotype
ts/hypersens	No growth at high temperature
dom	No growth (not viable)
ts/hypersens dom	No growth (not viable)
ts/hypersens dom	Growth at high temperature No growth at low temperature

F-15 / 19

Structurally Similar Compounds in lot N 94

F-15 / 11

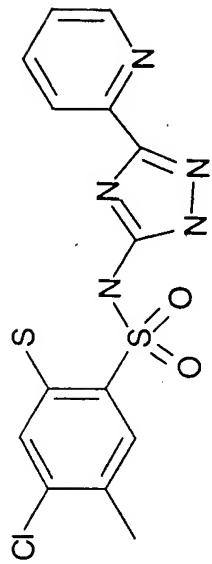
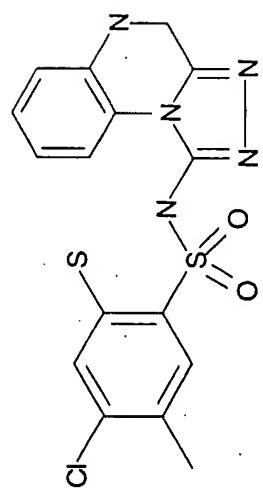
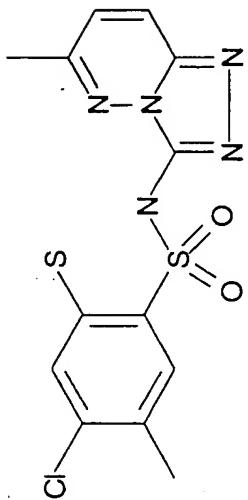
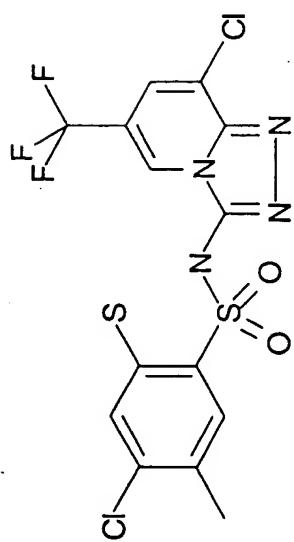


Fig. 20



Clone 8135-10m 1 HZev
8/21/96

Fig 21 NT3

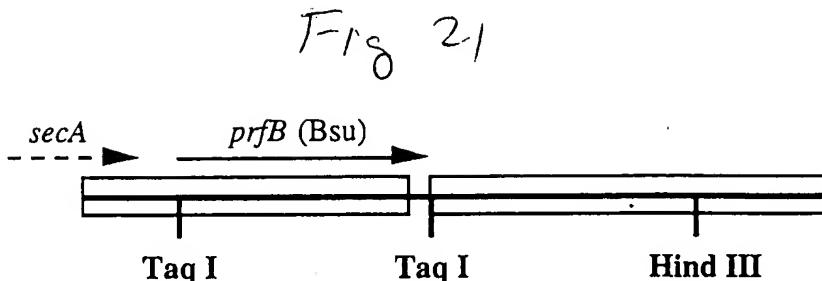


Fig 22 NT5

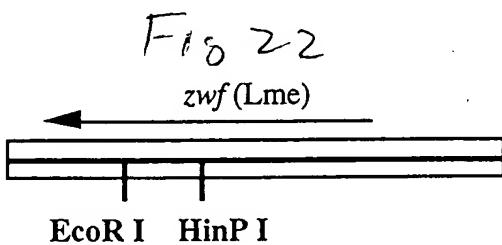


Fig 23 NT6

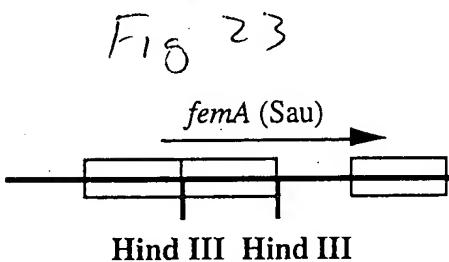


Fig 24 NT8

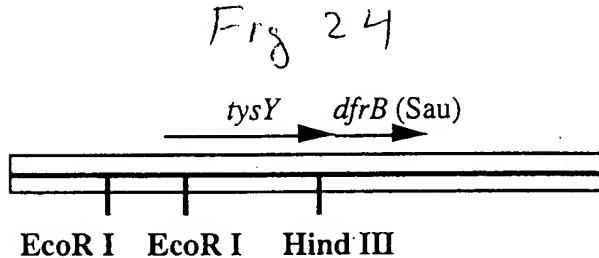
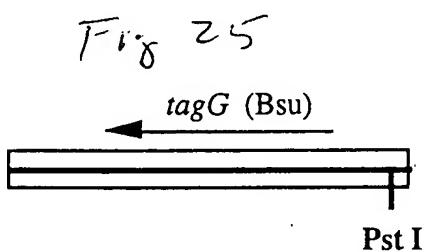
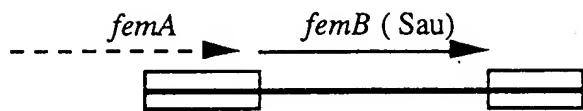


Fig 25 NT12



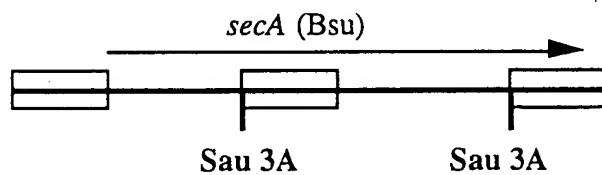
26 NT14

Fig 26



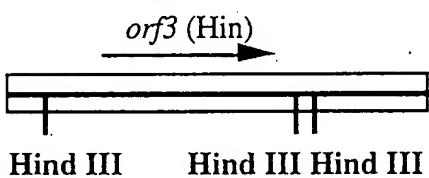
27 NT15

Fig 27



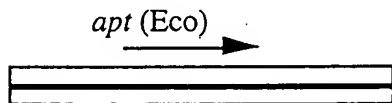
28 NT16

Fig 28



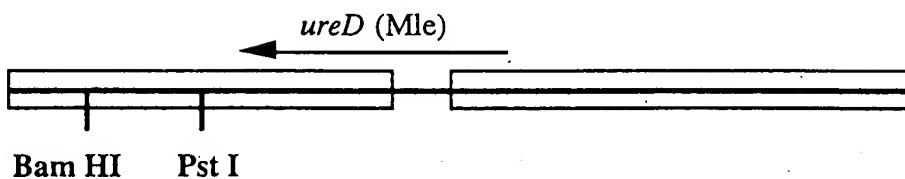
29 NT17

Fig 29

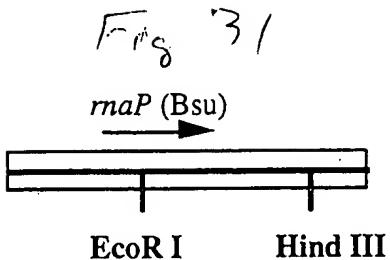


30 NT18

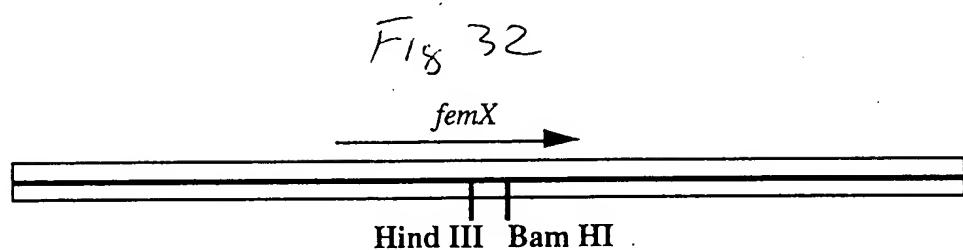
Fig 30



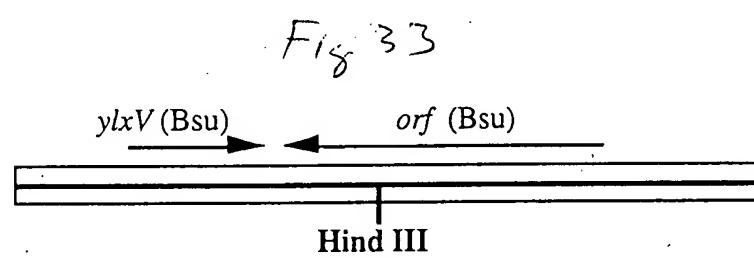
NT19



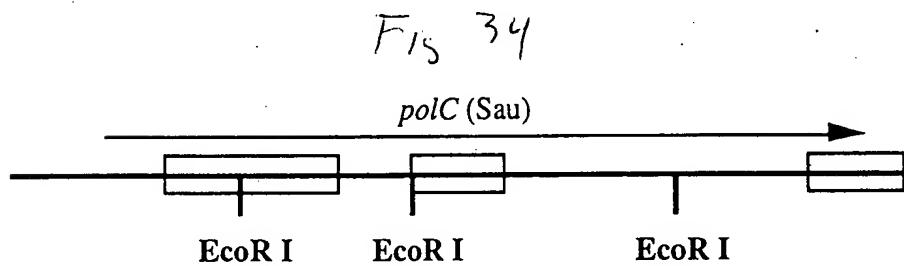
NT23



NT27

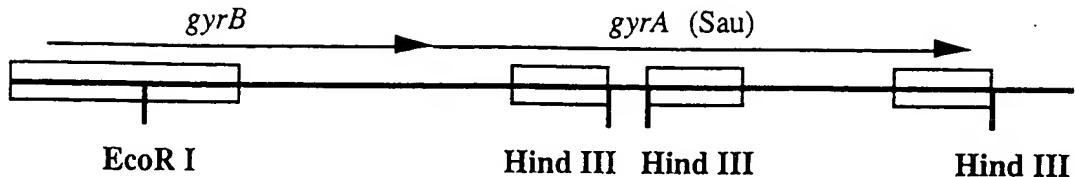


NT28



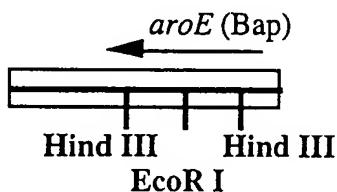
NT29

*F*₁₈ 35



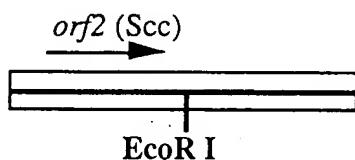
36 NT31

*F*₁₈ 36



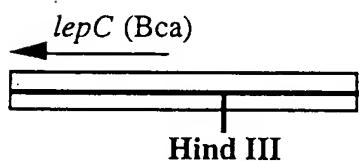
NT33a

*F*₁₈ 37



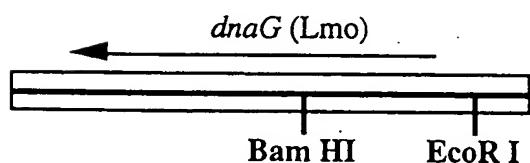
NT33b

*F*₁₈ 38



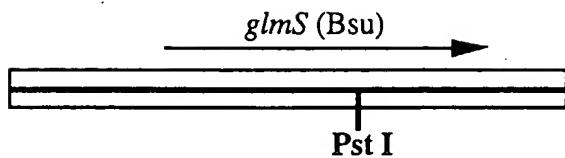
NT36

*F*₁₈ 39



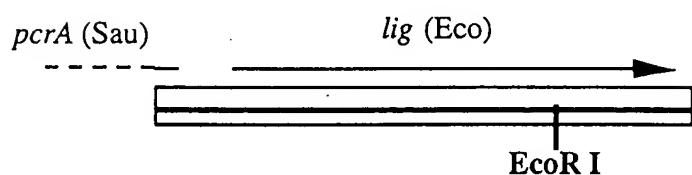
40 NT37

Fig 40



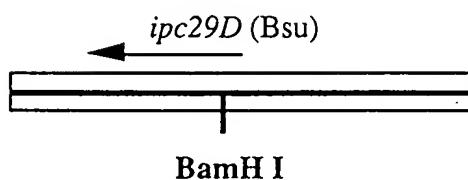
41 NT41/64

Fig 41



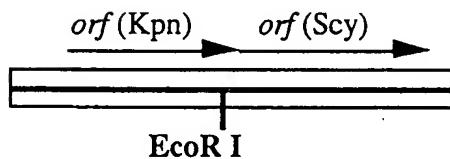
42 NT42

Fig 42



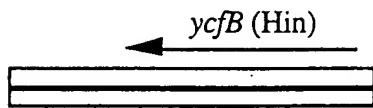
43 NT47

Fig 43



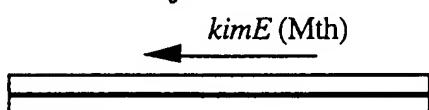
44 NT51

Fig 44



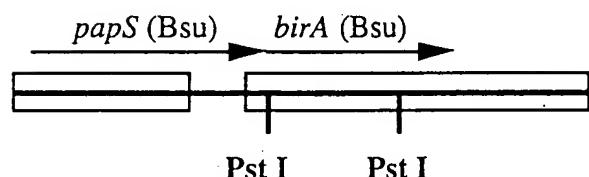
45 NT52

Fig 45



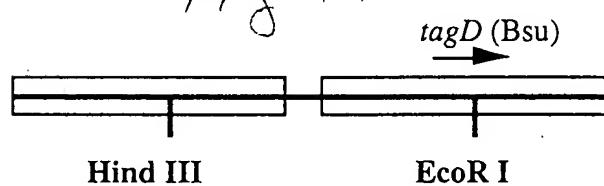
46 NT53

Fig 46



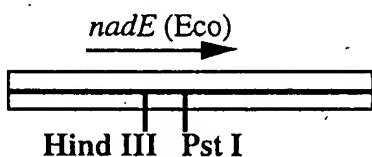
47 NT54

Fig 47

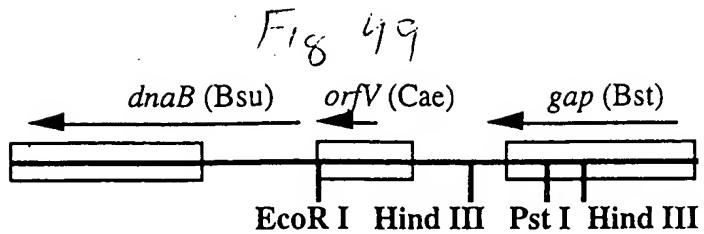


48 NT55

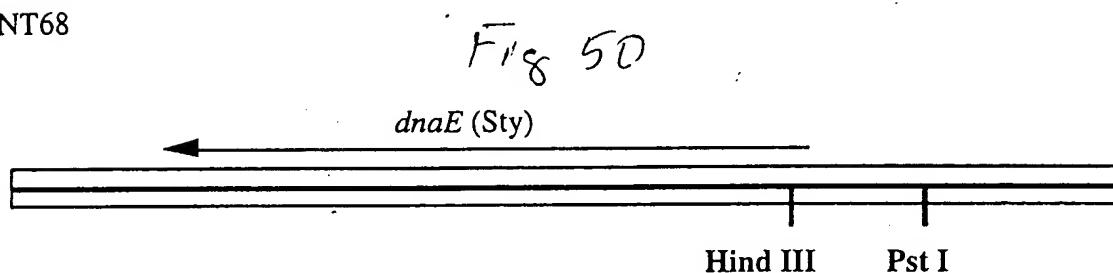
Fig 48



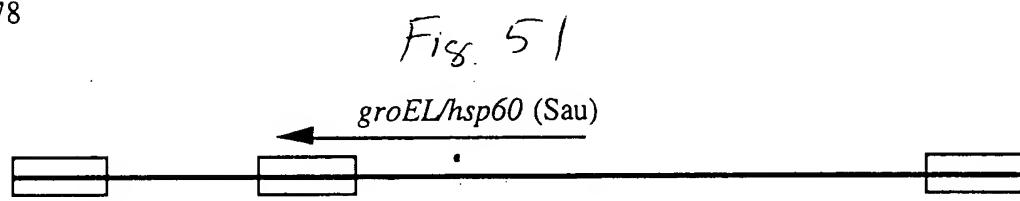
Li G NT57



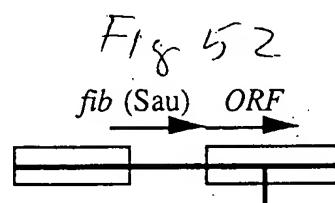
2 NT68



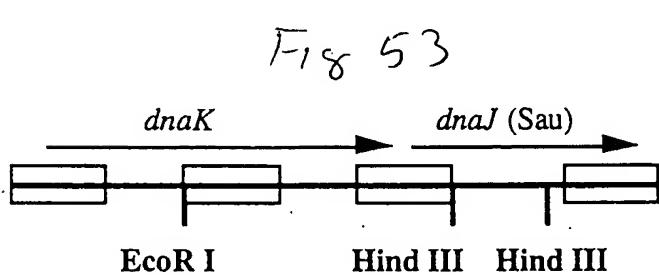
2 NT78



2 NT81



3 NT86



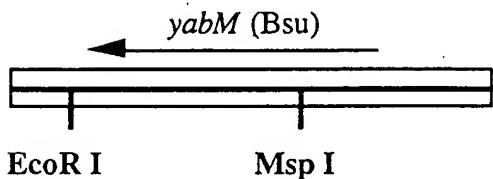
NT89

Fig 54



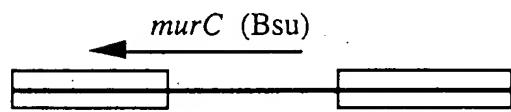
NT94

Fig 55



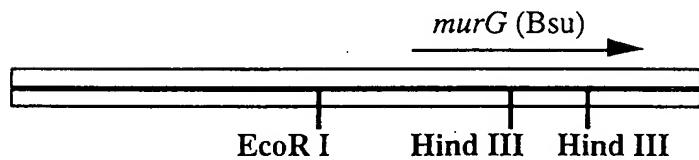
NT96

Fig 56



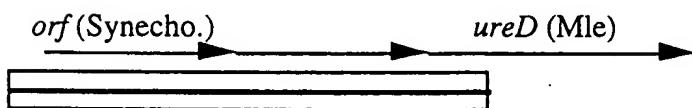
NT99

Fig 57



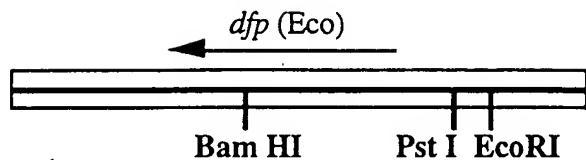
NT102

Fig 58



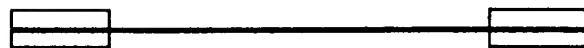
NT114

Fig 59



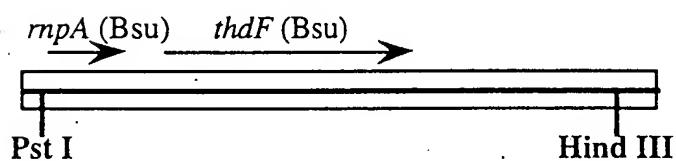
60 NT124

Fig 60



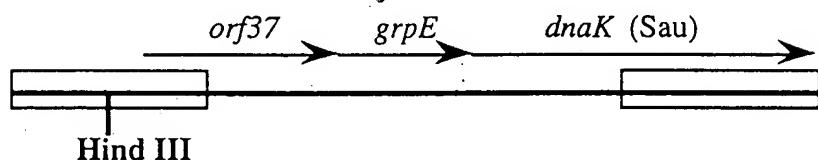
61 NT125

Fig 61



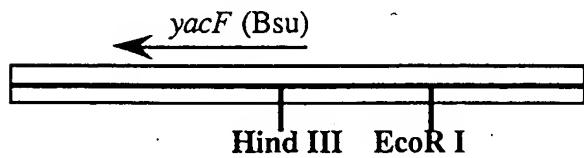
62 NT144

Fig 62



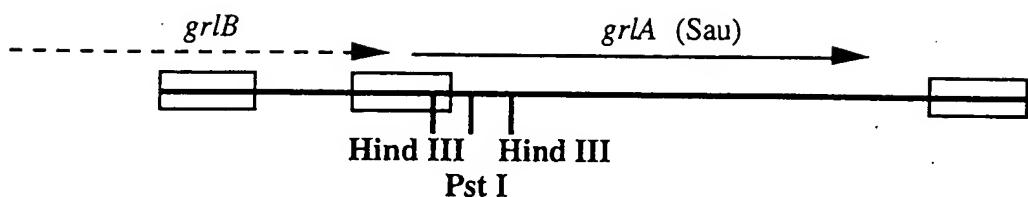
63 NT152

Fig 63



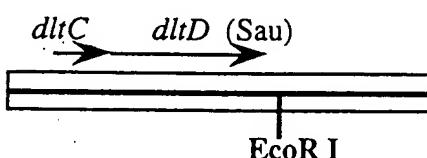
64 NT156

Fig 64



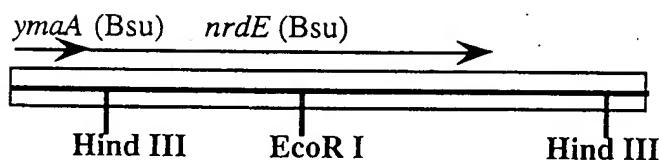
55 NT160

Fig 65



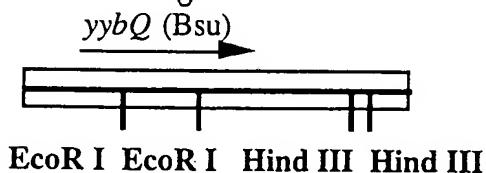
NT166

Fig 66



NT199

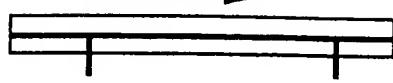
Fig 67



8 NT201

Fig 68

murB (Bsu)



Hind III

EcoR I

69 NT304

Fig 69

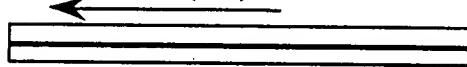
dod (Bsu)



70 NT310

Fig 70

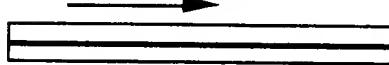
ddlA (Ehi)



71 NT312

Fig 71

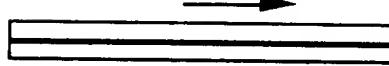
mg442 (Mge)



72 NT318

Fig 72

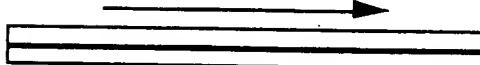
spoVC (Bsu)



73 NT321

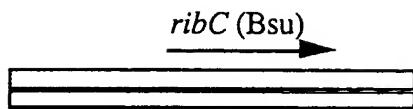
Fig 73

orf (Mtb)



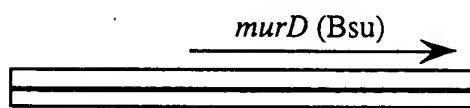
74 NT325

Fig 74



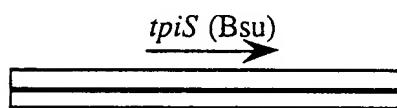
75 NT333

Fig 75



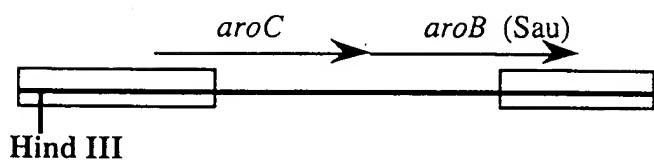
76 NT346

Fig 76



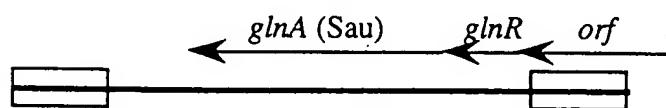
77 NT348

Fig 77

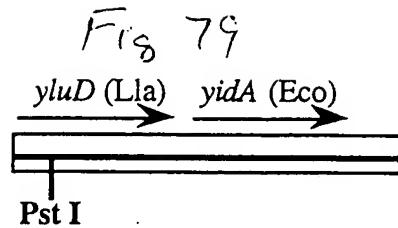


78 NT359

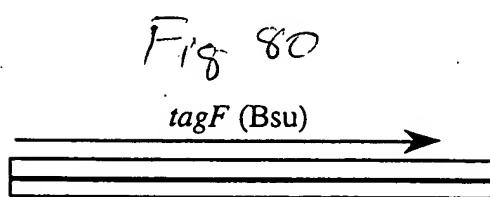
Fig 78



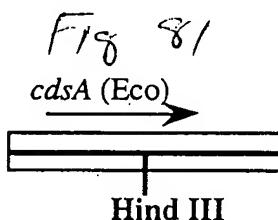
79 NT371



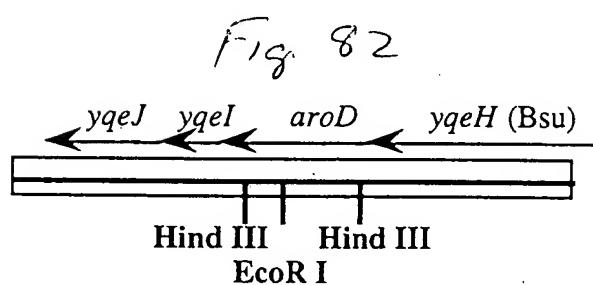
80 NT379



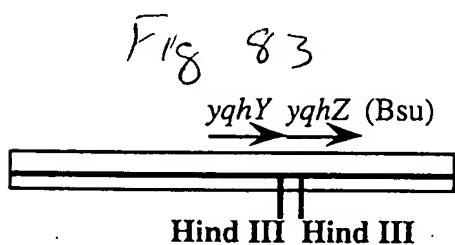
NT380



NT401

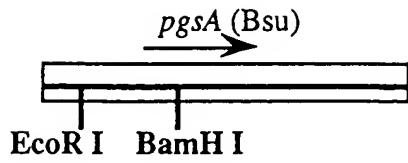


NT423



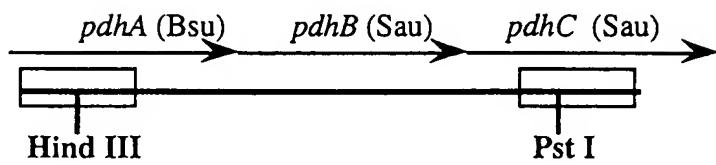
NT432

Fig 84



85 NT435

Fig 85



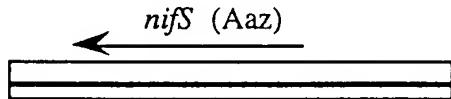
86 NT437

Fig 86



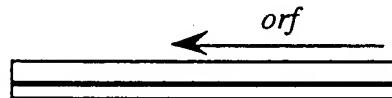
87 NT438

Fig 87



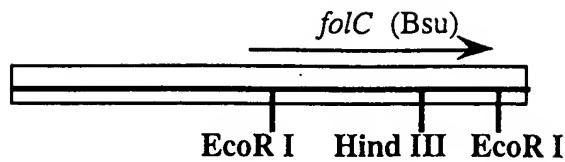
88 NT462

Fig 88



89 NT482

Fig 89



NT486

Fig 90

